1199-Plat
Remodeling of Actin Filaments by Cofilin
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Cofilin/ADF proteins play key roles in the dynamics of actin. We used cryo-electron microscopy of uniformly decorated actin-cofilin filaments to show that the cofilin induced change in the filament twist is due to a unique conformation of the actin molecule unrelated to any previously observed state. The changes between the actin protomer in naked F-actin and in the actin-cofilin filament are greater than the conformational changes between G- and F-actin. Cofilin/ADF proteins efficiently depolymerize F-actin only when bound at low stoichiometry to actin filaments. We also used cryo electron microscopy to reveal the structure of F-actin decorated with sub-stoichiometric amounts of cofilin. Our results suggest that the structural state of actin protomers found within the uniformly decorated actin-cofilin filaments can propagate towards the naked regions, and this cooperative propagation is uncoupled from the change in the helical twist of F-actin upon interaction with cofilin. This illustrates the structural plasticity of actin and provides a structural mechanism for actin depolymerization by the ADF/cofilin proteins.

1200-Plat
Counterion-Induced Formation of Regular Actin Bundle Networks
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Dominating the cytoskeletal contribution to cell mechanics and migration, actin and actin-crosslinker systems have attained much attention with regard to rheology and network architecture. In contrast to protein crosslinkers or molecular motors -which self-assemble and self-organize actin into networks, bundles or asters- simple multivalent ions rely on the polyelectrolyte nature of actin and should not imply any particular binding geometry. Using controlled counterion condensation as a model linker at comparably high F-actin densities we report an entirely novel state displaying regularly spaced networks of actin bundles connected by aster-like clusters. Liquid crystalline effects at high densities of long filaments were found to substantially alter the bundle network structures directly reflecting the pre-existing order of the actin filaments.

1201-Plat
Redundancy and Cooperativity in the Mechanics of Compositely Crosslinked Cytoskeletal Networks
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The actin cytoskeleton contains many types of crosslinkers—for example, crosslinkers that allow for free rotations between filaments and others that constrain the angle between crossing filaments. The mechanical interplay between different crosslinkers is an open issue in cytoskeletal mechanics. We develop a theoretical framework to study such a compositely crosslinked actin network to address this issue. We use an effective medium theory and computational modeling to carry out our investigations, with very good agreement between the two.
We find that the addition of angle-constraining crosslinkers, acting cooperatively with freely-rotating crosslinkers, allows the network to attain mechanical rigidity (a nonzero shear modulus) as soon as there exists a connected percolating cluster. This finding suggests the optimal conditions for the actin cytoskeleton to become rigid and be able to bear and transmit forces with the minimal amount of material possible. Without angle-constraining crosslinkers, the rigidity percolation threshold requires a higher density of filaments such that the transmission of forces is not as efficient. We further demonstrate that the introduction of angle-constraining crosslinks in flexible filament networks results in mechanical response similar to just freely-rotating crosslinked semiflexible filaments indicating redundancy. Our results also impact upon tissue engineering by providing design principles for developing filamentous scaffolds with tunable mechanical properties.

1202-Plat
Symmetry Breaking for Actin-Based Motility : From Molecular to Macroscopic
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Cells use their dynamic actin network to control their mechanics and motility. These networks are made of branched and growing filaments. Here we study under which conditions such networks are able to build up a stress and display mechanical properties. Hard beads coated with a nucleating promoting factor are placed in a minimal protein system containing actin, the Arp2/3 complex, Capping Protein, and profilin. We parallelize experiments and simulations and find that gel heterogeneities observed around the beads do not always lead to symmetry breaking and comet formation, due to the molecular structure of the network and how it grows. Actin gel morphology around the beads is directly related to the balance between filament nucleation and capping. We propose a predictive phase diagram linking the molecular and physical properties of growing actin gel. Therefore, in motile cells, a small change in protein concentration can thus lead to a dramatic change in cell mechanics and motility.

1203-Plat
F-Actin Mediated Chromosome Transport
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F-actin meshwork contraction is a novel mechanism of intracellular transport that was recently shown to transport chromosomes during meiosis in starfish oocytes. The starfish model system is well suited to high-resolution fluorescence microscopy imaging. We use a high-throughput volume filtering procedure as well as optical flow calculations and spatio-temporal image correlation spectroscopy (STICS). We determine the meshwork pore sizes, node distributions, and branching angles, and relate these structural features to predictions of the recently-proposed ''homogeneous contraction'' model of transport. We also perform indirect structure analysis by simulating diffusing particles migrating though the F-actin meshwork to probe local F-actin structures and to investigate how F-actin bundle remodeling or binding to F-actin bundles would affect particle motion. We analyze these results in the context of percolation theory and well-understood diffusion model systems. We furthermore compare the simulated particle trajectories to experimental observations of chromosomes and inert PEG-coated beads diffusing within both active and utrophin-stabilized F-actin meshworks. The experimental data are in good agreement with our simulations, and both suggest that chromosome-sized particles can be transported by the F-actin meshwork as passive cargo undergoing confined diffusion.

1204-Plat
Regulation of Actin Isoforms by Nitric Oxide
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Nitric oxide is a known regulator of cellular function that can act indirectly via activation of guanylyl cyclase, or directly via the S-nitrosylation of cysteine residues. However, little is known about the breadth of proteins that are regulated. Actin is present in nearly all eukaryotic cells and in a variety of isoforms that differ slightly in amino acid sequence, and more specifically in the number and placement of cysteine residues. It has been shown by others that different cysteines in the various isoforms can be modified by a number of oxidation reactions including S-nitrosylation. We therefore sought to determine the effect of S-nitrosylation on the interactions of skeletal, smooth, and non-muscle actins with myosin. We measured skeletal, smooth, and non-muscle (β and γ) actin nitrosylation in response to in vitro treatment with nitroso-L-cysteine - an endogenous nitrosothiol and NO donor - as well as actin filament velocity over heavy meromyosin (HMM), and the actin activated ATPase rates of HMM. A coumarin switch assay showed that all three isoforms could be nitrosylated by nitroso-L-cysteine. Nitrosylation of each actin isoform significantly decreased the in vitro velocity over HMM by approximately 38% with 50 μM donor compared to untreated controls. In contrast, there was no difference observed in the α-skeletal actin activated ATPase rates of the control and treated filaments. Further studies are being conducted to determine which cysteine residues in skeletal, smooth, and non-muscle actin are modified by nitroso-L-cysteine. Together these data suggest that nitrosylation of actin affects the attached time of actomyosin crossbridges. Given the ubiquitous nature of actin it is possible that nitrosylation is a common regulatory scheme for muscle contraction and various forms of cell motility.

1205-Plat
Force Transmission in a Reconstituted Actomyosin Cortex
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The cellular cortex is a dense, quasi 2D network of actin filaments coupled to the inner leaflet of the cell membrane and underlies numerous morphological and physical behaviors of cells, including division, migration and adhesion. The mechanical properties of the actin cortex determine how forces generated from myosin II motors are transmitted to facilitate contraction and tension build up at cellular (10-30 um) length scales. We assembled a reconstituted actomyosin cortex which serves as a model system to understand the roles of actin filament length, actin cross-linking proteins and actin-membrane adhesion to elucidate the mechanisms of cortex force transmission. We form a quasi-2D network of actin filaments of variable length near the surface of a supported

lipid bilayer and control the actin-actin or actin-lipid interactions by the addition of cross-linking proteins. Upon the addition of myosin II motors, we observe the movement of actin and myosin by timelapse confocal microscopy. In the absence of adhesion to the membrane and actin cross-linking proteins, contractility at 10-100 um length scales is only observed for sufficiently long (10 um) actin filaments. To facilitate contraction of short filaments (~ 1 um), the addition of an actin cross-linker protein is required. Increasing adhesion to the lipid bilayer reduces the rate of contraction while facilitating tension build up. Our results demonstrate the roles of actin network connectivity and membrane adhesion in modulating the nature of force transmission in a biomimetic model of the actin cortex.

Platform: Membrane Receptors & Signal Transduction II

1206-Plat

Biochemical Crosslinking and Liquid Chromatography-Mass Spectrometry Demonstrate a Rhodopsin Dimerization Interface Mediated by Helices 1 and 8

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Heptahelical G protein-coupled receptors (GPCRs) can exist as monomers and a ternary complex of ligand, GPCR and heterotrimeric G protein is the basic signaling unit. GPCRs also tend to form dimers and higher-order oligomers in membranes, although the functional consequences of these interactions are in most cases unknown. Furthermore, the precise protein-protein interface(s) in receptor dimers and the mechanisms mediating dimerization remain controversial. Two-dimensional and three-dimensional densities obtained from electron microscopy, as well as X-ray data on packing of rhodopsin crystals, suggest that the primary dimer contacts involve transmembrane helix (H) 1 and cytoplasmic H8. Biochemical crosslinking studies with dopamine D2 receptors hint that this interface may be broadly significant. We crosslinked rhodopsin dimers in native rod outer segment disk membranes to demonstrate the proximity of H8 between adjacent receptors. Four homobifunctional cysteine-reactive crosslinkers were used, two with maleimide groups and two with methanethiosulfonate groups. The formation of dimers and oligomers was verified by SDS-PAGE, size-exclusion chromatography, and immunoblot analysis. We used partial proteolysis and high-resolution liquid chromatography-mass spectrometry (LC-MS) to identify the site of a crosslink between Cys316-Cys316. Cys316 is one of two reactive cysteines in rhodopsin and is located in H8. The spacer length of the crosslinkers that formed the intermolecular Cys316-Cys316 crosslink is consistent with the distance predicted in a H1/H8 dimer model. This result corroborates findings from coarse-grained molecular dynamics (CGMD) potential of mean force (PMF) calculations, which show that the H1/H8 orientation is by far the most stable among the possible dimer orientations tested. Together these results strongly suggest the existence of this interface in native membranes. Given the high degree of homology across class A GPCRs, these results may be relevant for other receptors.

1207-Plat

The Retinal Energy Landscape as a Function of the Rhodopsin Photocycle

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Retinal is the covalently bound inverse-agonist of the prototypical G protein-coupled receptor, rhodopsin. It comprises a β-ionone ring and polyene chain covalently bound to Lys296 of rhodopsin by a protonated Schiff base (PSB). During the course of rhodopsin activation, retinal initially undergoes an 11-*cis* → all-*trans* isomerization, followed by a deprotonation of the Schiff base. Using quantum chemical calculations at the MP2 level of theory and solid state NMR spectroscopy we demonstrate substantial differences in retinal structure and dynamics between the protonated and deprotonated species. The delocalization of positive charge from the PSB results in perturbations of the entire retinal moiety upon deprotonation. For example, methyl rotation barriers are shifted as much as 200% [1]. Surprisingly, deprotonation of retinal drastically affects the energetics of β-ionone ring rotation, producing an extra minimum in the C5=C6-C7=C8 torsional energy surface. This results in a proton affinity (PA), and hence pK_a, that depends on β-ionone ring orientation. Specifically, the PA of retinal is lowered for non-planar conformations of the β-ionone ring, in turn lowering the pK_a and facilitating the deprotonation required for formation of the Meta I pre-activated state of rhodopsin. In order to extend these calculations to account for interactions within the rhodopsin binding pocket we have used the QM data, including MP2 level torsion scans of every dihedral angle, to refine new retinal force fields for both protonation. This data opens the door to future molecular dynamics studies of retinal proteins that include the activated Meta II state. [1] B. Mertz *et al*. (2011) *Biophys. J.* **101**: L17.

1208-Plat

Activation Mechanism of the β2-Adrenergic Receptor

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A third of marketed drugs act by binding to a G protein-coupled receptor (GPCR) and either triggering or preventing receptor activation. While recent crystal structures have provided snapshots of both active and inactive functional states of GPCRs, these structures do not reveal the mechanism by which GPCRs transition between these states. Here we characterize the activation mechanism of the β_2-adrenergic receptor, a prototypical GPCR, using the first simulations in which a GPCR transitions spontaneously from one crystallographically observed state to another (Nature 469:236 (2011); PNAS, in press); the total duration of these all-atom simulations, at over 600 microseconds, is unprecedented for a molecular system of this size. A loosely coupled allosteric network, comprising three regions that can each switch individually between multiple distinct conformations, links small perturbations at the extracellular drug-binding site to large conformational changes at the intracellular G protein-binding site. Our simulations also reveal an intermediate that may represent a receptor conformation to which a G protein binds during activation, and suggest that the first structural changes during receptor activation take place on the intracellular side of the receptor, far from the drug-binding site. By capturing this fundamental signaling process in atomic detail, our results may provide a foundation for the design of drugs that control receptor signaling more precisely by stabilizing specific receptor conformations.

1209-Plat

Dynamic Monomer-Dimer Equilibrium of a Prototypical GPCR, Beta2 Adrenergic Receptor: A Single Molecule Imaging Study

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Recently, by developing special single-molecule techniques and analysis theory, we first time ever succeeded in obtaining the two-dimensional association equilibrium constant in the membrane and fully characterizing the equilibrium by also obtaining both dissociation and association rate constants (Kasai et al., 2011). As an important paradigm for this work, we examined the monomer-dimer equilibrium of a GPCR, formyl-peptide receptor (FPR). Here, we applied this method to a prototypical class-A GPCR, beta2-adrenergic receptor (B-AR), to clarify whether it forms dimers. Although B-AR has been studied extensively, whether it (and other class-A GPCRs in addition to FPR) exists as dimers has been controversial. We found that B-AR does form dimers with rate and equilibrium constants similar to those for FPR (FPR values in parentheses): the monomer-dimer equilibrium constant = 1.6 (3.6) copies/square microns; the dimer dissociation rate constant of 12.6 (11.0) /s [dimer lifetime of 80 (90) ms]; and the monomer association rate constant of 7.9 (3.1)/[copies/square microns]/s. However, under physiological conditions, the behaviors of B-AR and FPR were quite different due to the difference in the expression levels, ~260 and ~2.1 copies/square microns [740,000 (6,000) copies/cell] in dog heart cells and neutrophils, respectively. The majority (95%) of B-AR would exist as dimers, whereas about half (42%) of FPR would do so. Interestingly, their dimer lifetimes are similar, ~80 and ~90 ms, respectively, but their monomer lifetimes would be vastly different, i.e., 0.49 ms and 154 ms, respectively. These results suggest that the dynamic monomer-dimer equilibrium might play a role in signal transduction and/or its regulation.

1210-Plat

Interactions of CCR5, the Main HIV Coreceptor, with Rantes and Other Ligands

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The entry of the human immunodeficiency virus 1 (HIV-1) into host cells requires the sequential interaction of the viral envelope glycoprotein 120 (gp120) with the host-cell factor CD4 and with either CCR5 (CC chemokine receptor 5) or CXCR4, both G-protein coupled receptors (GPCR). This leads to the fusion of viral and host cell membranes. The normal physiological